

SYSTEMS AND METHODS FOR PRODUCTION OF RECOMBINANT IL-11 IN YEAST

[0001] This application claims the benefit of U.S. Provisional Application No. 62/446,762, filed on Jan. 16, 2017. These and all other referenced extrinsic materials are incorporated herein by reference in their entirety. Where a definition or use of a term in a reference that is incorporated by reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein is deemed to be controlling.

FIELD OF THE INVENTION

[0002] The field of the invention is production and subsequent purification of recombinant IL-11, particularly in yeast.

BACKGROUND

[0003] The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0004] The interleukin IL-11 has considerable therapeutic potential, however production of IL-11 at adequate scale and purity has proven challenging. Due to the lack of glycosylation expression of recombinant IL-11 in bacteria has been attempted. The resulting protein, however, tends to be expressed as insoluble inclusion bodies, resulting in poor yields. This is probably due to improper folding. Attempts have been made to express IL-11 in yeast, however to date such processes have provided low yields and have required the use of toxic organic solvents.

[0005] One approach to address this is to express recombinant IL-11 as a fusion protein having more desirable expression characteristics. Commercially available IL-11 is typically isolated from a fusion protein expressed in *E. coli*. Unfortunately the use of enterokinase to generate an IL-11 fragment from the fusion protein results in product heterogeneity. Similarly, United States Patent Application Publication No. 2009/0010872 (to Mackiewicz) describes a recombinant IL-11 fusion protein that incorporates both IL-11 and soluble IL-11 receptor sequences and expression of such a fusion protein in insect or mammalian cells in culture. Recovery of IL-11 from such a fusion protein, however, requires additional processing steps that cleave the fusion protein and can result in variations in the length and/or sequence of the product IL-11 fragment. In addition such cells have complex culture requirements that can complicate downstream purification of the desired product.

[0006] For example United States Patent Application Publication No. 2007/0275889 describes the use of a plasmid encoding for both an IL-11 sequence and a chaperonin, and expression of such a plasmid in insect or mammalian cells in culture. The chaperonin serves to provide proper folding and prevent aggregation of the expressed IL-11. All publications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that

term provided herein applies and the definition of that term in the reference does not apply. As noted above, however, culture conditions for such cells can complicate subsequent purification steps. In addition, expression in mammalian and insect cells in culture is generally far lower than that of bacteria or yeast.

[0007] Thus, there is still a need for a simple, effective, and scalable method for providing substantially pure and active IL-11.

[0008] In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0009] As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

[0010] Unless the context dictates the contrary, all ranges set forth herein should be interpreted as being inclusive of their endpoints, and open-ended ranges should be interpreted to include only commercially practical values. Similarly, all lists of values should be considered as inclusive of intermediate values unless the context indicates the contrary.

[0011] The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value with a range is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0012] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the speci-